Bonn. zool. Beitr.	Bd. 50	H. 1–2	S. 35–48	Bonn, Dezember 2001

New bioacoustic records of *Rana bedriagae* Camerano, 1882 (Anura: Ranidae) from Turkey

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Abstract. The advertisement calls of water frogs at nine sites along the south coast and within Turkey were recorded, analyzed and compared with those of *Rana ridibunda* Pallas, 1771, and *R. bedriagae* Camerano, 1882. All the call characteristics, whether temperature-dependent or not, are consistent with those of *R. bedriagae* but not those of *R. ridibunda*. It follows that these localities are inhabited by Levantine frogs, *R. bedriagae*.

Key words. Advertisement calls, comparative call analysis, distribution, *Rana bedriagae*.

Introduction

Until the past decade the Lake frog, *Rana ridibunda*, was regarded as a species with a very broad distribution. Large parts of Europe, Central Asia and the Near East were thought to be included in its range, as well as the Arabian peninsula and parts of the Nile Valley (Günther 1990, 1991). The contributions towards changing this picture was mainly made by bioacoustic studies, particularly in eastern Mediterranean regions. Comparative studies of the advertisement calls showed that in Israel, Egypt, Syria, and western and southern Turkey the Levantine frog, *Rana bedriagae*, is found (Nevo & Schneider 1983, Schneider & Sofianidou 1985, Joermann et al. 1988, Schneider 1997a, 1999a, Schneider & Sinsch 1999). The existence of *R. bedriagae* as a distinct species was confirmed by comparative allozyme analyses and electrophoretic studies (Nevo & Filippucci 1988, Sinsch & Eblenkamp 1994), as well as by differences in morphological characters (Sinsch & Schneider 1999).

R. ridibunda is also native to regions bordering the eastern Mediterranean Sea and the Black Sea. The results of bioacoustic studies indicate that it lives in Thrace (Greece), at Burgas in Bulgaria, in the Danube delta and in Armenia: the advertisement calls of these frogs are consistent with those of the Lake frogs in Atyrau (Kazakhstan), the type locality of this species (Schneider & Sofianidou 1985, Schneider & Egiasarjan 1989, 1991, Günther et al. 1991, Schneider & Sinsch 1992, Schneider 1997b).

Turkey has been considered part of the range of *R. ridibunda* as well (Bodenheimer 1944, Mertens 1952, Basoğlu & Özeti 1973, Baran 1981, 1984, Yilmaz 1984, Kasparek & Kasparek 1990, Leviton et al. 1992). Based on bioacoustic findings (Joermann et al. 1988), however, it was concluded that the populations at İzmir and Dalaman represent another form, which later was identified as *R. bedriagae* (Schneider & Sinsch, 1999). According to recent bioacoustic and morphometric studies (Schneider & Sinsch 1999, Sinsch & Schneider 1999) *R. bedriagae* also lives at Alanya, on the Karpuz Çayı in the south and in Beyşehir Gölü in inner Turkey. On the other hand, the water frogs of Beyşehir Gölü were described by Arıkan (1988) as a new subspecies *R. ridibunda caralitana*, and the authors of many very recent morphological studies on frogs from various regions of Turkey came to the conclusion that they were dealing with *R. ridibunda* (Atatür et al. 1989/1990, Arıkan 1990, Baran 1990, Baran et al. 1992, Arıkan et al. 1994, Kaya 1996, Baran & Atatür 1998, Arıkan et al. 1998, Tok 1999, Kumlutaş et al. 1999, Tok et al. 2000, Budak et al., 2000). Finally, Jdeidi et al. (2001) followed our conclusion that *R. bedriagae* occurs in Beyşehir Gölü and found this species also at other places.

Here we present new bioacoustic information to assist in clarifying the taxonomy of the water frog fauna of Turkey.

Materials and methods

In 1998 (12–27 June), 1999 (17–24 May) and 2000 (21–31 May), series of advertisement calls of water frogs were sampled at nine localities in Turkey (Fig. 1): Yakaköy (N=7), Eğirdir (N=21), Dimçayı (N=5), Gazipaşa (N=9), Tenzile (N=5), Örenbucağı (N=13), Taşucu (N=11), Ortaköy (N=3) and Ereğli (N=5). The calls were recorded with a condenser microphone (Sennheiser, Type MKH 415 T) and a portable tape recorder (Stellavox SP 8), and the corresponding water temperature with an electrical thermometer (Technoterm 1500). Oscillograms (Tektronix Oscilloscope 502 A, Toennies Recordine camera) were prepared for evaluation and the depicted parameters measured. Each call series was characterized by nine

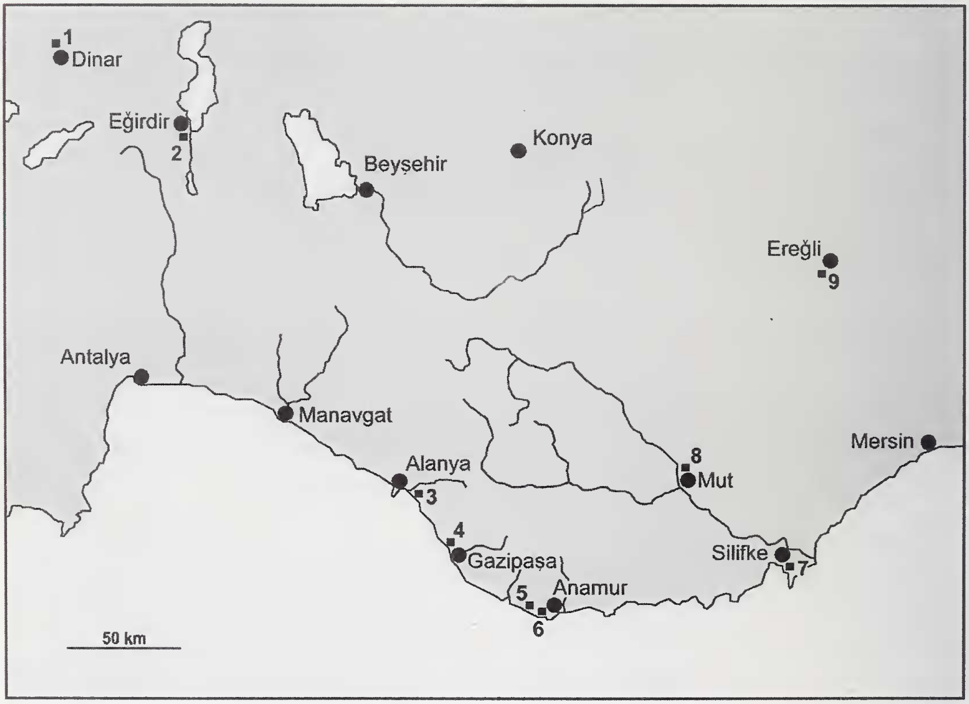


Fig. 1. The geographical locations at which advertisement calls had been recorded: 1 Yakaköy, 7 km northwest of Dinar, 2 Eğirdir, 3 Dimçayı, 4 Gazipaşa, 5 Tenzile, 6 Örenbucağı, 3.8 km west of Anamur, 7 Taşucu, 8 Ortaköy, 4 km north of Mut, 9 Ereğli.

parameters: 1. duration of pulse groups [ms]; 2. interval between pulse groups [ms]; 3. period of pulse groups [ms]; 4. call duration [ms]; 5. intercall interval [ms]; 6. call period [ms]; 7. number of pulse groups/call [N]; 8. pulses/pulse group [N]; 9. pulse groups/s [Hz]. The arithmetic means of these call parameters were calculated for each series and used for further analyses. Thus, the basic data set describing the advertisement calls of each population consisted of 10 variables (9 call parameters and the corresponding temperature) with N (the number of call series) observations.

These calls were compared with those recorded at other localities in the Middle Eastern states Turkey, Syria, and Israel and had been identified as either *Rana ridibunda* or *R. bedriagae* (Schneider & Sinsch 1999). Series of the advertisement calls of lake frogs *R. ridibunda* from Kazakhstan, Armenia and Greece (Schneider & Sinsch 1992) were also used for comparisons.

At the level of local populations, the four temperature-dependent call parameters (pulse group duration, pulse group interval, pulse group period, pulse groups per second; Schneider & Sinsch 1992) were plotted against water temperature in order to detect potential differences in the type of dependence. The small range of temperatures and the low number of call series impeded a statistical comparison among localities based on regression lines. In contrast, the five temperature-independent call parameters (pulses per pulse group, pulse groups per call, call duration, intercall interval, call period; Schneider & Sinsch 1992) were statistically compared by means of a one-way ANOVA for each variable. Grouping of populations was determined by a multiple range test using the Bonferroni method with a confidence interval of 99%.

At the level of species (reference populations and new populations), a principal-component analysis was performed on the matrix of the linear correlation coefficients of all individual variables in order to reduce the amount of information to statistically unrelated factors. The first principal component (PC1) derived from advertisement call data represents the amount of variance caused by temperature, the following ones (PC2/PC3) temperature-independent sources of variation. The influence of temperature on PC1 was assessed by linear correlation and regression analysis for each species, and slopes and intercepts of regression lines were tested for species-specific differences, using the conditional sum of squares. Finally, a discriminant function based on the five temperature-independent variables was obtained to distinguish between *R. ridibunda* and *R. bedriagae* (Schneider & Sinsch 1999); this was used to classify the calls of the frogs of the nine new localities.

Results

Habitats and calling behaviour

The water frogs under study are widely distributed in southern Turkey. They live in all kinds of running and stagnant waters, on the banks of rivers, in little streams, creeks, artificial channels, lakes, pools, artificial water reservoirs, and in swamps or flooded areas rich in vegetation. The coloration of the frogs is highly variable. In many frogs the dorsum is green with irregular dark green spots, while other animals have brown dorsal coloration. The vocal sacs of the males are dark grey or black.

During the time of observation, in May and June, the male frogs exhibited a high level of calling activity. Calling began in the morning, was low or absent during the afternoon, and continued vigorously for several hours after sunset. Advertisement calls, three types of territorial call, and release calls could be heard. Calling activity occurred over a large temperature range. For example, the lowest water temperature at which calling was recorded occurred in the Dimçayı which flows from a mountain source high in the Taurus Mountains Range. Here the males called at water temperatures as low as 13.3°C. In contrast, frogs in a pond at the outskirts of Alanya at the southern coast, frogs called at water temperatures as high as 33.5°C.

Statistical evaluation of advertisement calls

The advertisement calls exhibit a typical structure. The main feature is that they are made up of pulse groups (Fig. 2). Depending on the ambient water temperature several call parameters change dramatically. As the oscillograms show, at low water temperatures the pulses of the pulse groups as well as the pulse groups are clearly recognizable because the repetition rate of the pulses is low and long intervals separate the pulse groups. At high temperatures the pulse-repetition rate is high resulting in short pulse groups, and the intervals between the pulse groups are very short (Fig. 2).

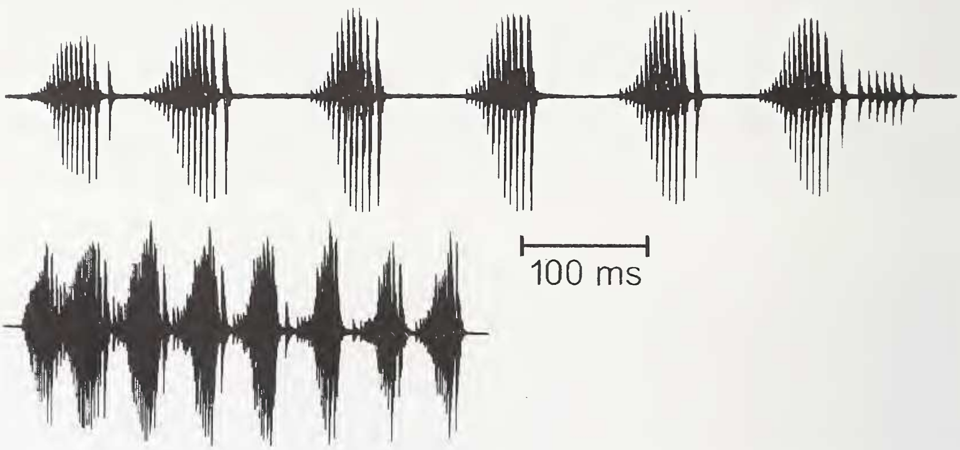


Fig. 2. Oscillograms of two advertisement calls recorded at 13.3°C (top) and 24.5°C (bottom) water temperature.

Previous analyses of the advertisement calls of *R. bedriagae* and *R. ridibunda* demonstrated that the call variables of pulse groups per second, duration of pulse groups, interval between pulse groups and period of pulse groups significantly varied with the ambient temperature (Schneider & Sinsch 1992, 1999). This was also true for the water frog populations in this study (Fig. 3). In any population with call recordings at different temperatures, the expected positive or negative correlation of each call variable with temperature was obvious, e.g. in the populations 1 (Yakaköy), 6 (Örenbucaği) and 9 (Ereğli).

The five temperature-independent call variables showed geographical trends in some cases (Fig. 4). There was a slight local variation in the number of pulse groups per call (ANOVA, P=0.019), but following Bonferroni correction of confidence intervals all populations formed one homogeneous group (Multiple range test, P>0.05). In contrast, populations considerably differed in the number of pulses per pulse group from west to east (ANOVA, P<<0.0001). The multiple range test resolved three distinct groups (significance level: P<0.05): calls recorded at Yakaköy formed the

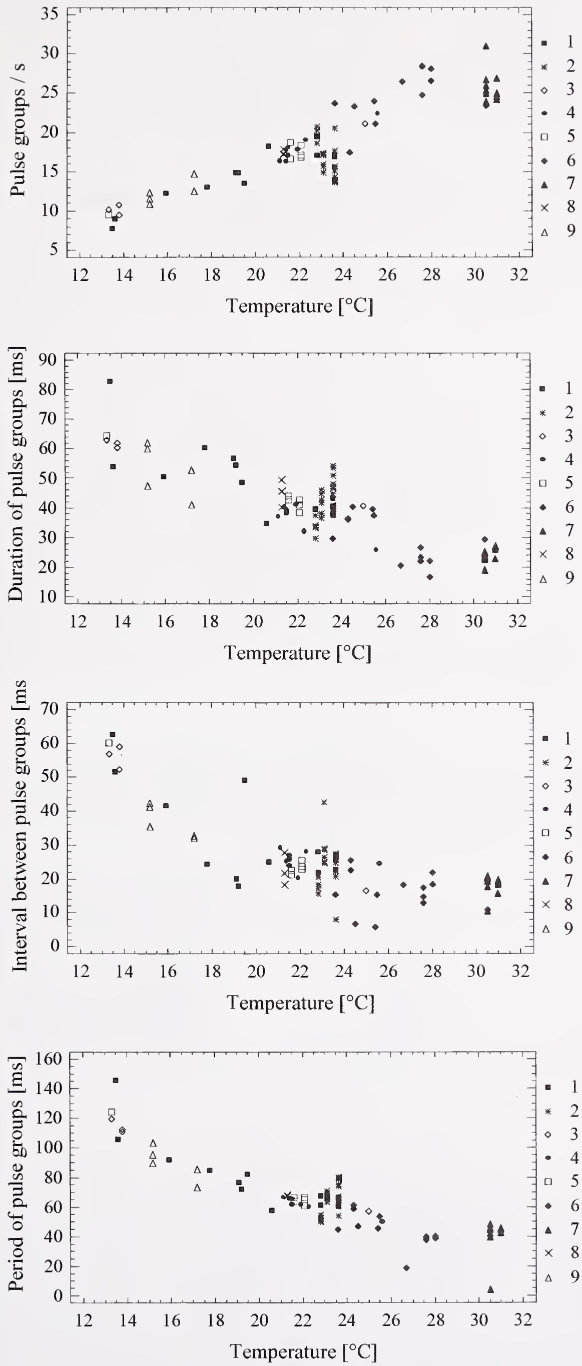
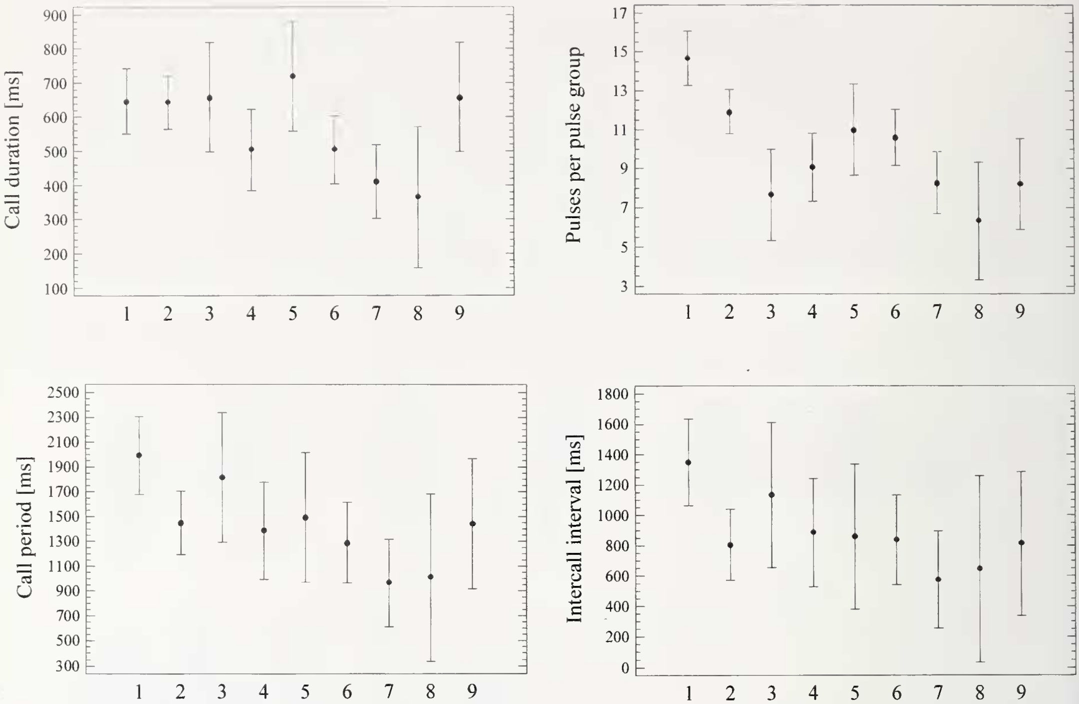


Fig. 3. Temperature dependence of four advertisement call variables in nine water frogs populations in Turkey. The geographical distribution of localities is shown in Fig. 1.



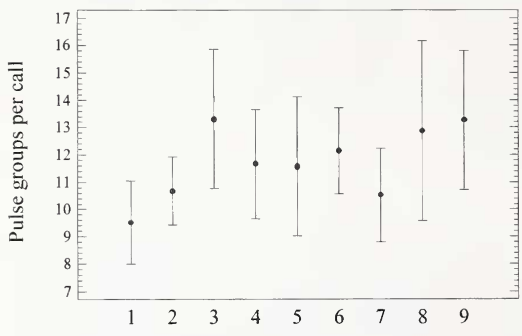


Fig. 4. Temperature-independent variation of five advertisement call variables in nine water frog populations in Turkey. Means and corresponding 95% confidence interval. Statistics in Table 1. The geographical distribution of localities is shown in Fig. 1.

first group, those recorded at Eğirdir the second one, and the calls recorded at the other seven sites the third homogeneous group. The call duration did not show a clear geographical trend, but differed locally (ANOVA, $P=0.0001$). Two homogeneous groups were detected, one with a mean call duration of 500 ms or less, the second with 640 ms or more (Multiple range test, $P<0.05$). The intercall intervals were very variable within each population and also between populations (ANOVA, $P=0.007$). Therefore, there were no well-resolved groups. Only population 1 (Yakaköy) significantly differed from 2 (Eğirdir) and 7 (Taşucu; Multiple range test, $P<0.05$). Due to the pattern of variation in call duration and intercall interval, the call period also failed to show interpretable geographical trends, but differed locally (ANOVA, P=0.0003). Again, the only significant differences detected were between population 1 (Yakaköy) on one side and 6 (Örenbucaği) and 7 (Taşucu; Multiple range test, P<0.05) on the other side.

Taxonomic identification of advertisement calls

The univariate analyses of nine call variables provided evidence that there is local variation of call structure among the nine populations studied. However, variation did not reach a level that would suggest the presence of different taxa within these populations of water frogs. Therefore, we pooled all data and used multivariate analyses to compare this sample with those of *R. bedriagae* and *R. ridibunda* (Schneider & Sinsch 1999). Principal component analysis of the complete data set (9 call variables + temperature, 516 observations) showed that a significant influence of temperature on the call structure was represented in component 1 (Table 1, Fig. 5). The comparison of the linear regression models describing the temperature dependence of call structure revealed that the regression line obtained for the new populations significantly differed from that calculated for *R. ridibunda*, but was indistinguishable from that calculated for *R. bedriagae* (Tab. 2).

With respect to the temperature-independent component of call structure we used the discriminant function which significantly distinguishes between *R. bedriagae* and

Table 1: Principal component analysis of the pooled data set (9 call variables + temperature) on the advertisement calls recorded in Turkey for this study (N=78), and the reference calls of *R. bedriagae* (N=217) and of *R. ridibunda* (N=221, see Schneider and Sinsch, 1999).

a) Principal components extracted (eigenvalue > 1):

Component Number	Eigenvalue	Percent of Variance	Cumulative Percentage
1	6.03	60.3	60.3
2	1.62	16.2	76.6
3	1.03	10.3	86.8

b) Component weights:

	PC 1	PC 2	PC 3
temperature	+0.3491	+0.0305	+0.1503
pulse groups/s	+0.3844	+0.1014	+0.1688
duration of pulse groups	–0.3693	–0.1537	–0.0328
interval between pulse groups	–0.3492	+0.0037	–0.1903
period of pulse groups	–0.3905	–0.0718	–0.1302
number of pulse groups/call	+0.2107	+0.5475	–0.3562
pulses/pulse group	–0.2982	–0.2708	+0.1105
call duration	–0.2181	+0.4444	–0.5069
intercall interval	–0.2431	+0.3974	+0.6069
call period	–0.2820	+0.4812	+0.3608

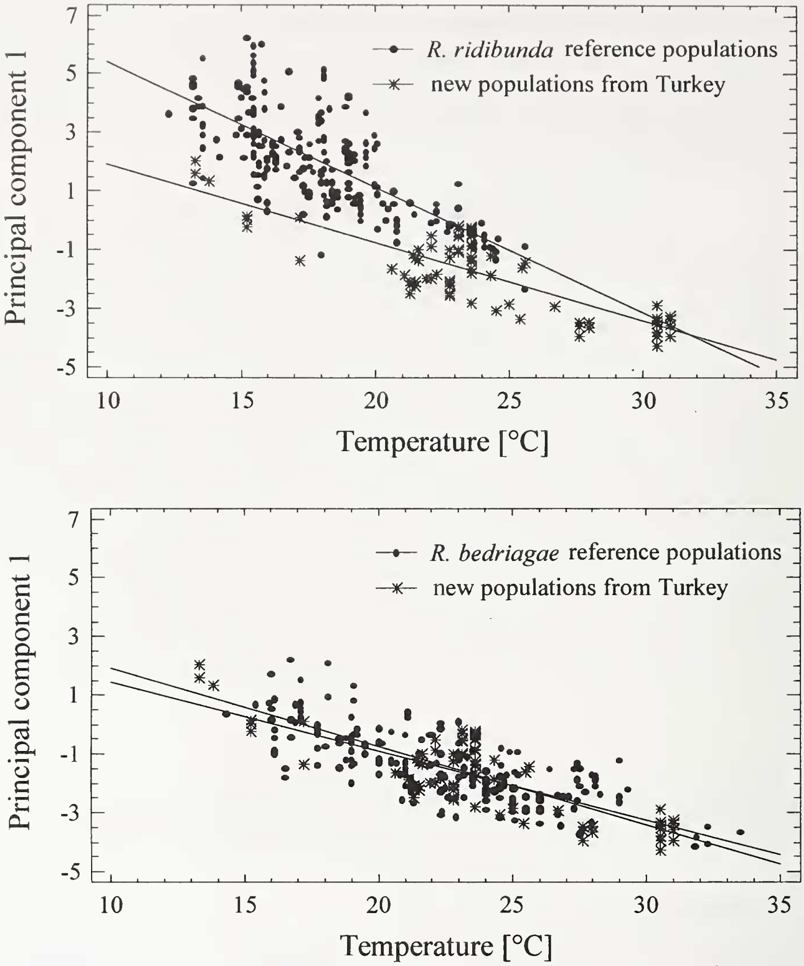


Fig. 5. Temperature-dependent advertisement call variation (presented as principal component 1 based on nine call variables, Table 1) in nine water frog populations from Turkey in comparison to that of *Rana ridibunda* (top) and *R. bedriagae* (bottom) reference populations. Regression lines significantly differed between *R. ridibunda* and the new populations with respect to slopes and intercepts, but not between *R. bedriagae* and the new populations (Table 2).

R. ridibunda (Schneider & Sinsch 1999) to compute the discriminant scores for the new populations from Turkey. The range of discriminant scores for this group completely overlapped with that of *R. bedriagae*, whereas 82 % of scores fell outside the range of *R. ridibunda* (Fig. 6). Still, medians of the frequency distribution also differed between the new populations and *R. bedriagae* (u-test: $P<0.01$) reflecting the local call variations already detected in the univariate analyses.

Table 2: Statistical comparison of linear regression models which describe the influence of temperature on principal component 1, i.e. the temperature-dependent component of the advertisement call structure (Fig. 6).

a) Linear regression models:

Group	slopes ± SE	intercepts ± SE	R^2
R. ridibunda	−0.48±0.03	10.74±0.49	59.1%
R. bedriagae	−0.30±0.01	5.26±0.29	72.2%
New populations	−0.33±0.02	6.04±0.47	78.8%

b) Two groups (*R. ridibunda*, new populations) comparison using ANOVA:

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Temperature	1369.47	1	1369.47	1250.32	<<0.0001
Intercepts	93.52	1	93.52	85.39	<<0.0001
Slopes	17.58	1	17.58	16.05	0.0001
Model	1480.57	3			

c) Two groups (*R. bedriagae*, new populations) comparison using ANOVA:

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Temperature	466.71	1	466.71	852.02	<<0.0001
Intercepts	0.17	1	0.17	0.31	0.58
Slopes	0.99	1	0.99	1.80	0.18
Model	467.86	3			

Discussion

The results of the statistical calculations indicate that the advertisement calls of the water frogs living at nine sites in the south of Turkey constitute a uniform sample. It follows that these frogs belong to a single taxon. Furthermore, the statistical results also demonstrate that these frogs represent Levantine frogs, *R. bedriagae*, because their advertisement calls are consistent with those of the Levantine Frogs in the immediate vicinity of Damascus, the type locality of *R. bedriagae*, as well as with those of the Levantine frogs in Israel, the Nile delta and five sites in Turkey (Nevo & Schneider 1983, Schneider & Sofianidou 1985, Akef & Schneider 1989, Schneider & Sinsch 1992, 1999, Schneider et al. 1992, Schneider 1997a, 1999a).

The identification of the water frogs in the Middle East as *R. bedriagae* and the conclusion that they are distinct from *R. ridibunda* are based not only on the advertisement calls but also on the results of allozyme analyses (Sinsch & Eblenkamp 1994) and of morphometric investigations (Sinsch & Schneider 1999). Morphometric examination of frogs from several sites in Syria and Jordan indicates that the range of *R. bedriagae* includes all of Syria and Jordan.

The new bioacoustic studies in the south of Turkey expand the range of *R. bedriagae* still further, beyond the localities already known in this country: İzmir, Dalaman, Karpuz Çayı at Haciobaşi, Alanya, Beyşehir Gölü. Although no reliable

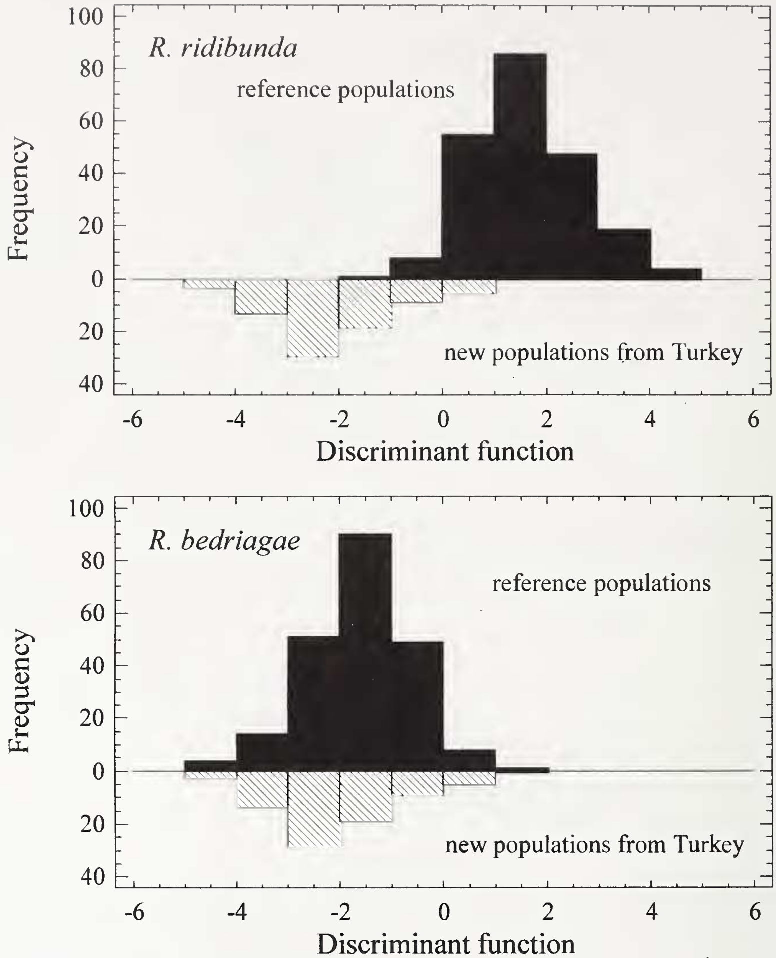


Fig. 6. Temperature-independent advertisement call variation in nine water frog populations from Turkey in comparison to that of *R. ridibunda* (top) and *R. bedriagae* (bottom) reference populations. Data are presented as frequency histograms (class width: 1 unit) of discriminant scores which were calculated using the function to distinguish between *R. ridibunda* and *R. bedriagae* (Schneider & Sinsch 1999).

data are available yet for the region between Ereğli, the easternmost site in the new studies, and Syria, it is highly probable that *R. bedriagae* is likewise native to the extreme southeast of Turkey. Recently Franzen & Schmidtler (2000) studied the distribution and ecology of *Triturus vittatus* in southern Turkey; they mentioned that *R. bedriagae* also occurs at some sites, but gave no information that a special method was used to identify this species.

Because all of Turkey was formerly claimed to belong to the range of *R. ridibunda*, the authors of many faunistic, morphological and morphometric studies assumed that they were dealing exclusively with *R. ridibunda*. Having visited 66 islands in the northern Aegean, the Sea of Marmara and the western Black Sea, Baran (1981) reported that *R. ridibunda* was present there in considerable numbers. Yilmaz (1984), during an investigation of the amphibian fauna of Turkish Thrace, found *R. ridibunda* at 59 sites. Arıkan (1990) determined the nuclear DNA content and used morphological and serological methods to identify water frogs from four geographically distant localities of Turkey. With respect to the nuclear DNA content the frogs from Ulubey at the eastern coast of the Black Sea differed from those of the other three localities, the region of the lakes at Beyşehir, the region of Marmara and of Malatya in the central eastern part. All frogs were assigned to *R. ridibunda* assuming that the differences are on the level of populations. According to Baran et al. (1992), *R. ridibunda* occurs along the Black Sea coast between the Sakarya and Yeşılirmak, and according to Kumlutaş et al. (1999), it also lives on the eastern coast of the Black Sea. In addition, Tok (1999) showed that *R. ridibunda* inhabits the Datça peninsula, and Tok et al. (2000) found *R. ridibunda* at Dalaman on the south coast, a locality that according to Schneider & Sinsch (1999) is inhabited by *R. bedriagae*. As Tok et al. (2000) further report, most of the water frogs in this region are distinguished by black and yellow warts on the underside. Such features are also typical of the water frogs near the source of the Sakarya at Çifteler (personal observation). Arikan (1988) established a subspecies of *R. ridibunda*: *Rana ridibunda caralitana* Arıkan, 1988; the type locality is the Beyşehir Gölü. The crucial distinguishing feature of these frogs was the conspicuous orange markings on the ventral parts of the body. *R. r. caralitana* is also found in the Eğirdir Gölü, Suğla Gölü and Çarsamba Suyu, including its tributaries (Atatür et al. 1989/1990) as well as at many sites between Isparta and the Hotamiş lakes (Arikan et al. 1994), at İvriz-Ereğlı (Arikan et al. 1998) and in the Işkli Gölü (Budak et al. 2000). Only very recently did opinion change. Jdeidi et al. (2001) made a morphometric study of water frogs from various regions in Turkey including Beyşehir Gölü and agreed with our conclusion based on bioacoustic and morphometric results (Schneider & Sinsch 1999, Sinsch & Schneider 1999) that *R. bedriagae* occurs in Turkey. With respect to the subspecies *caralitana* Jdeidi et al. (2001) suggest specific status. The advertisement calls of the frogs at Beyşehir Gölü are distinguished by low dominant frequency and differ in this regard from those of the Levantine frogs in other localities – for instance the frogs that live at Alanya (Schneider 1999b). The difference in the spectral composition of the advertisement calls, however, is secondary. The frogs at the Beyşehir Gölü are exceptionally large, and hence the structures involved in generating the calls are large, which causes the sounds to be low-pitched. This size-related effect on the advertisement calls would not suggest a special taxonomic status. The orange coloration on the ventral surface of these frogs is indeed a striking feature. Whether it suffices as a distinguishing characteristic of a subspecies seems questionable, particularly since the coloration of the water frogs in Turkey is extremely variable. It is worth mentioning that Alpagut & Falakalı (1995) analyzed the karyotype of water frogs from Beyşehir and İzmir and found differences in certain chromosomes.

R. bedriagae probably occupies a large part of Turkey, but *R. ridibunda* may also be present. The verified findings of *R. bedriagae* in the southern area of the range previously ascribed to *R. ridibunda* indicate that *R. bedriagae* seems to be a species adapted to high temperatures. One of the main factors responsible for the distribution of the lake frog, *R. ridibunda*, in the northern parts of Europe and Asia is probably that it has adapted to low winter temperatures. This is indicated by the decidedly continental climate in large areas of the range, for example in Armenia and at the type locality Atyrau in western Kazakhstan (Schneider & Egiasarjan 1989, 1991, Schneider 1997b). Further evidence is the fact that *R. ridibunda* lives in Thrace, the eastern province of Greece (Schneider & Sinsch 1992). The climatic conditions here are appreciably less favourable than in the western parts of Greece. Given that *R. ridibunda* is present in the Greek part of Thrace, it seems evident that this species should also be native to neighbouring Turkish Thrace, as Yilmaz (1984) reported. The presence of *R. ridibunda* in Armenia suggests that the range of this species might also extend into the northern and eastern parts of Turkey.

The question of which part of Turkey is occupied by *R. bedriagae* demands further study.

Zusammenfassung

Paarungsrufe von Wasserfröschen wurden an neun Standorten an der Südküste und im Landesinneren der Türkei aufgezeichnet, analysiert und mit denen von *Rana ridibunda* Pallas, 1771, und *R. bedriagae* Camerano, 1882 verglichen. Sowohl die temperatur-abhängigen als auch die temperatur-unabhängigen Merkmale der Paarungsrufe stimmen mit denen von *R. bedriagae* der Typuslokalität Damaskus, Syrien, überein, nicht aber mit denen von *R. ridibunda* der Typuslokalität Atyrau, Kasachstan. Daraus folgt, dass an den ausgewählten Lokalitäten Levantefrösche, *R. bedriagae*, vorkommen.

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